Aus dem Diabetes-Forschungsinstitut der
Universität Düsseldorf
Direktor: Prof. Dr. Hans Reinauer

Effects of Anabolic Steroids on the Alkaline Proteolytic Activity in Skeletal Muscles of Normal and Endurance Trained Rats

Dissertation
zur Erlangung des Grades eines Doktors der Medizin
der Medizinischen Fakultät der Universität Düsseldorf

vorgelegt von
Albertus Budiharta Widjaja

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Synopsis

Administration of testosterone propionate or metenolone acetate (10 mg/kg body wt., twice a week for 8 weeks) to male rats resulted in a 10-30% decrease of the alkaline proteolytic activity in musculus soleus and musculus extensor digitorum longus. This alteration of activity was observed irrespective of the reference value chosen (protein, DNA, muscle wet weight). Endurance swimming training for 8 weeks lead to an increase of about 50% of the alkaline proteinase activity in musculus extensor digitorum longus and about 10% in musculus soleus of normal rats as compared to sedentary control rats. This exercise mediated increase of proteolytic activity was prevented by simultaneous administration of 10 mg testosterone propionate/kg body wt. twice a week. Injection of the same amount of metenolone acetate to exercising rats was less effective in suppressing the proteolytic activity. The data suggest that the anti-catabolic action of testosterone is mediated not only by its regulatory effect on protein synthesis but also by alteration of the protein degradative mechanism.

The hormonal dependence of protein metabolism in skeletal muscle is well documented (Cahill et al., 1972; Goldberg, 1969; Flaim et al., 1978 a,b). Besides several other hormones such as insulin, glucocorticoids, growth hormone and thyroxine, testosterone is a predominant regulator for growth rate of muscles (Kochakian, 1966), in so far as testosterone and related androgenic steroids have protein anabolic effects (for review see Kochakian, 1976). Additionally, it is known that testosterone has an anticatabolic effect (for review see, Kochakian, 1976).

In a previous study we have shown that depletion of testosterone by testectomizing male rats lead to an increase in muscle alkaline proteolytic activity. Subsequent treatment of the animals with the hormone decreased the activity to control values (Dahlmann et al., 1980). These findings suggested that the hormones' regulatory function in the control of muscle proteolytic activity could be an important aspect of its anticatabolic properties. However, since testectomy per se might have consequences for the endocrine status of the animals, the more general validity of our hypothesis was tested by extending the experiments on castrated rats to healthy, non-castrated rats. Thus, testosterone administration would be expected to lead to a reduction of basal levels of muscle alkaline proteolytic activity.

In another approach, animals were subjected to endurance training, which is known to decrease serum testosterone levels and increase urea nitrogen excretion (Dohm & Louis,

1978; Dohm et al.,1977b). Consistent with our hypothesis, there should be a concomitant increase in muscle alkaline proteolytic activity which should be reversed by testosterone administration.

Materials and Methods

Male rats (Wistar HaN strain) purchased from Winkelmann, Paderborn, Germany, were fed a standard diet (Sniff, Intermast, Soest, Germany) and water ad libitum.

Azocasein and deoxyribonucleic acid from calf thymus were products of Serva, Heidelberg, Germany. Testosterone propionate (Testoviron) and metenolone acetate (17ß-hydroxy-1-methyl-5-androst-1-en-3-one) were obtained from Schering AG, Berlin, Germany.

Muscle preparation

Rats were sacrificed and musculus soleus and musculus extensor digitorum longus were prepared from hind limbs (Goldberg et al., 1975). The muscles were weighed and then homogenized in 10 vol. (w/v) of 1 M-KCl/ 0.05 M-Tris/HCl/ 0.2 % (v/v) Triton X-100, pH 7.4. The homogenization procedure and preparation of the muscle crude extract was performed as described elsewhere (Dahlmann et al., 1980).

Measurement of proteolytic activity

The overall alkaline proteolytic activity was tested with 3 % (w/v) azocasein as the substrate according to the described procedure (Dahlmann & Reinauer, 1978). One unit

is defined as the amount of enzyme producing a change of absorbance at 366 nm of 0.001/min.

Measurement of protein was done by the biuret method using LAB-TROL (Dade, Miami, Fl., U.S.A.) as a standard. DNA concentration was determined as described by Afting & Elce (1978) but omitting the pronase digestion step.

Protocol of experiment

30 rats were randomly divided into six groups each comprising five animals. Twice a week the animals received the following injections:

group N : 0.1 ml sesame oil/kg body wt.
group N_t: 0.1 ml sesame oil/kg body wt.
group T : 10 mg testosterone propionate/kg body wt.
group T_t: 10 mg testosterone propionate/kg body wt.
group M : 10 mg metenolone acetate/kg body wt.
group M_t: 10 mg metenolone acetate/kg body wt.

The animals of the groups N_t, T_t and M_t were subjected to swimming training. The training program consisted of two swimming peroids of 45 min each with a break of 10 min in water of 30°C. This exercise program was performed daily after the animals had become accustomed to the swimming: during the first five days the program was initiated with two swimming sessions each lasting 15 min and then increasing by 2 times 15 min/day. The training was omitted on days when the animals received hormone injections. The animals belonging to the sedentary groups N, T, and M were kept untrained

in their cages. Hormonal treatment and training were performed for 8 weeks. One day after the last exercise the animals were sacrificed and muscles were excised.

Results

Body weights

Treatment of untrained rats for two month with anabolic steroids lead to a delay in gain of body weight. As shown in Table 1 , this retardation was more pronounced in testosterone treated rats than in metenolone treated animals. Swimming exercise, however, enhanced this decrease in gain of body weight in the metenolone treated animals.

Alkaline proteinase activity

In both muscles of the untrained rats we found that treatment of the animals with anabolic steroids lead to a decrease of the alkaline proteolytic activity. This effect was observed irrespective of the reference value (muscle wet weight, protein, DNA) chosen to compare the activity. The reduction of activity was more pronounced in the muscles of the metenolone treated group than in those of the testosterone treated rats (Tables 2 and 3).

Swimming training of the rats resulted in a considerable change of alkaline proteolytic activity in these muscles: the activity was significantly elevated in the extensor digitorum longus of the trained groups N_t, T_t and M_t when compared to the untrained rats. The differences amounting to

50% between N_t and N, about 20 % between T_t and T and about 106 % between M_t and M were statistically highly significant in the untreated and metenolone treated groups, but the activities measured in the groups T_t and T were statistically not different (Table 2). On the other hand a comparison of the proteolytic activities within the groups of trained animals (N_t, T_t and M_t) shows a decrease of activity in the extensor digitorum longus due to anabolic steroid treatment. In this respect testosterone was more effective than metenolone (Table 2).

The alkaline proteolytic activity in the musculus soleus was also increased by swimming training but the effect was less pronounced as in the extensor digitorum longus. (Table 3). Similarly, suppression of this increase of enzyme activity by treatment with anabolic steroids was only observed in the testosterone treated group of animals whereas the proteolytic activities in the solei of the rats belonging to the N_t and M_t groups were identical.

Muscle weight, protein and DNA content

All these changes of proteolytic activity were observed irrespective of the reference value chosen to compare the activities. (The only exception is that the activity in the musculus soleus is not elevated by training, when DNA was used as reference value. Table 3) The small alterations of these parameters, i.e. wet weights of the muscles as well as their protei and DNA content, are given in Table 4 and Figures 1 and 2 ;

The wet weight of the extensor digitorum longus was slightly

increased in the trained rats compared to the untrained rats except for the metenolone treated group (Table 4). As shown in Fig.1 this is the only group in which the protein content of the extensor digitorum longus in the trained rats (M_t) was higher than in the untrained rats (M), although it was not different from the N value. The DNA content was reduced in the extensor digitorum longus of trained rats except for the group treated with metenolone acetate (Fig. 1). Elevated muscle weight/body weight ratios were found in all the trained groups when compared with the untrained rats, but the differences were statistically insignificant (Table 4).

The wet weight of the musculus soleus was slightly lower in animals treated with anabolic steroids (T_t, T, M_t,M) as compared to untreated rats (N,N_t) and training had no influence on this parameter (Table 4). Due to the decreased body weight of testosterone treated animals (Table 1) their musculus soleus weight/body weight ratio is slightly but statistically insignificantly elevated (Table 4). Neither exercise nor administration of anabolic steroids changed the protein content of the soleus, but the DNA content was higher in the muscle of trained animals when compared to the untrained rats (Fig. 2).

Discussion

After testosterone administration the overall alkaline proteolytic activity in muscle of healthy male rats is 10-20 % lower than in control animals. This reduction of acti-

vity was not due to changes in the protein content or in muscle weight of steroid treated animals, since the decreased activity was found irrespective of the reference value chosen. This reduction in activity was observed with both the soleus and the extensor digitorum longus.

Simple after-effects of the decreased proteinase activities are unlikely to have occurred, since neither muscle weight nor protein or DNA contents were significantly changed by steroid treatment. Possibly, simple weight measurement or protein content determinations are not sensitive enough methods to detect small alterations of protein metabolism in muscle.

Despite this, the data obtained in the present study agree well with the results obtained in our studies with castrated rats (Dahlmann et al., 1980). One has to mention, that the effect of testosterone on the muscle alkaline proteinase activity is more easily demonstrated after castration and subsequent testosterone administration. For example, when daily injections of testosterone propionate were given for 7 days only, no decrease in alkaline proteinase activity could be obtained, but extending the daily treatment to 17 days did produce a noticable effect (data not shown).

Dohm & Louis (1978) recently reported that plasma testosterone is lowered by about 50 % in male rats subjected to endurance training. Knowing that increased testosterone levels lower the activity of the muscle alkaline proteinases in rats and conversely, since testosterone depletion as a result of castration leads to a dramatic increase of the activity of these enzymes, one would expect, that endurance training of untreated rats entails in increased proteinase activities.

In fact our experiment with swimming training lead to a similar result. As shown in Table 2 and 3, we found increased alkaline proteolytic activities in the extensor digitorum longus and soleus of rats trained during 8 weeks. Interestingly, in the extensor digitorum longus this increase was about 50 %, whereas in the soleus the increase was less than 10 %. This larger difference could be explained at least in part by a fiber type specific response to a decreased testosterone level. For example, it is known that the catabolic action of glucocorticoids is more pronounced in pale fibers than in continuously active red fibers (Goldberg & Goodman, 1969). Therefore it seems reasonable to assume that lack of testosterone affects predominantly the extensor digitorum longus, a muscle containing 97 % fast fibers as opposed to the rat soleus containing only 16 % fast fibers (Ariano et al., 1973). Additionally, this increase of proteolytic activity seems to depend on the duration of the training, since a single one hour swimming exercise does not affect the muscle alkaline proteinase activity (Dohm et al., 1980).

On the other hand, testosterone administration to non-exercising rats decreased the proteolytic activity not only in the extensor digitorum longus but also in the soleus (Table 2 and 3). This indicates that both muscles are testosterone sensitive. Alternative explanation would be that the larger increase of proteolytic activity in the extensor digitorum longus as compared to the soleus in trained rats is a consequence of different training intensities.

Provided that the increase of alkaline proteolytic activity is the result of lowered serum testosterone level, this effect

should be neutralized when trained rats are injected with testosterone, which our data clearly show to be the case. The alkaline proteolytic activity in the extensor digitorum longus of rats of the group T_t was considerably lower when compared to N_t and was indistinguishable from the activity measured in the T rats. This anticatabolic effect of testosterone was observed in musculus soleus as well.

Although it is evident from the data obtained in our experiment that metenolone acetate like testosterone propionate suppresses the muscle alkaline proteolytic activity in untrained rats, this anabolic steroid appears to be a poor substitute for testosterones' ability for preventing an increase of proteolytic activity during endurance training. As shown in Table 3, the proteolytic activity in the soleus of group M_t does not differ from that found in the soleus of N_t; in the extensor digitorum longus of animals from group M_t the enzyme activity is only slightly reduced as compared to the activity in N_t (Table 2). These results suggest that the suppressive effect of anabolic steroids on the basal activity of muscle alkaline proteinases works by a mechanism different from their normalizing effect on the elevated proteolytic activity in muscle of trained rats.

It is worth mentioning that exercise leads not only to lower serum levels of testosterone but also of insulin, while growth hormone and cortisol hormone levels are increased (Hartley et al., 1972). From earlier investigations in this and other laboratories it is known that insulin decreases the muscle alkaline proteolytic activity and conversely,

that glucocorticoids have a stimulating effect on these proteinases (Mayer et al, 1974; 1976; Röthig et al., 1978; Dahlmann et al., 1979). Thus the regulation of muscle alkaline proteinase activity may be achieved by the concerted action of several hormones. On the other hand, we cannot at present exclude that various hormones regulate different proteolytic enzymes because the spectrum of the alkaline proteinases in rat skeletal muscle is largely unresolved (Pennington, 1977).

Irrespective of the regulatory mechanism, stimulation of the alkaline proteinase activity in the trained rats is in agreement with the findings of Dohm et al.(1977b) and Dohm & Louis (1978) that endurance training of rats is accompanied by an increased urea nitrogen excretion and a deterioration of the nitrogen balance. This is partly a consequence of a decreased protein synthesis which is not confined to muscle alone (Dohm et al.; 1977a), but,in addition, the increased alkaline proteolytic activity may enhance protein catabolism in muscle of rats subjected to endurance training. On the other hand, administration of anabolic steroids to exercising rats not only increases the muscle protein synthesis (Rogodzkin, 1979), but,as shown in the present report, suppresses the enhanced activity of muscle proteinases.

Considering these data and the results obtained with castrated rats (Dahlmann et al., 1980), it seems likely that the anticatabolic effect of testosterone and anabolic steroids on muscle is a result of both: an increase of protein synthesis and a decrease of the activity of proteolytic enzymes.

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Table 1 Changes in body weight

Body weights at the beginning and the end of the experimental period of control rats (N), rats treated with testosterone (T) and those treated with metenolone (M) without and with swimming training ($_t$), respectively. Means $\pm$ S.D. are given. Gain in mean body weights are given in gram.

Table 2 Alkaline proteolytic activity in musculus extensor digitorum longus

The alkaline proteolytic activity in musculus extensor digitorum longus of control rats (N), testosterone-treated rats (T) and metenolone-treated rats (M) are given and compared with the activities in those animals subjected to 8 weeks of swimming training ($_t$). Mean values $\pm$ S.D.are compared by Student's t-test. n.s., not significant ($p > 0.05$).

Table 3 Alkaline proteolytic activity in musculus soleus

The alkaline proteolytic activity in musculus soleus of control rats (N), testosterone-treated rats (T) and metenolone-treated rats (M) are given and compared with the activities in those animals subjected to 8 weeks of swimming training ($_t$). Mean values $\pm$ S.D. are given and compared by Student's t-test. n.s.,not significant ($p > 0.05$).

Table 4 Muscle weights and muscle weight/body weight ratios

Wet weight of musculus soleus and extensor digitorum longus as well as muscle weight/body weight ratio in control rats (N) and rats treated with testosteron (T) or metenolone (M) are compared with rats subjected to 8 weeks of swimming training ($_t$). Statistical significance of means $\pm$ S.D. was tested by Student's t-test. n.s., not significant ($p > 0.05$).

group of animals	body weight (gram)		
	initial	final	change
N	177 ± 3	305 ± 37	+ 128
N_t	185 ± 7	301 ± 26	+ 116
T	178 ± 9	254 ± 47	+ 76
T_t	183 ± 7	255 ± 20	+ 72
M	181 ± 9	297 ± 22	+ 116
M_t	181 ± 4	260 ± 7	+ 79

Table 1

group of animals	proteolytic activity		
	U/g wet wt.	U/mg protein	U/µg DNA
N	64.2 ± 5.0	0.35 ± 0.04	0.11 ± 0.01
N_t	96.7 ± 12.0	0.53 ± 0.05	0.21 ± 0.01
	$p < 0.001$	$p < 0.01$	$p < 0.001$
T	61.7 ± 7.9	0.34 ± 0.03	0.10 ± 0.01
T_t	74.1 ± 21.2	0.40 ± 0.09	0.14 ± 0.04
	n.s.	n.s.	n.s.
M	43.3 ± 13.1	0.26 ± 0.07	0.07 ± 0.02
M_t	89.2 ± 10.9	0.46 ± 0.05	0.16 ± 0.04
	$p < 0.001$	$p < 0.01$	$p < 0.01$

Table 2

group of animals	proteolytic activity		
	U/g wet wt.	U/mg protein	U/µg DNA
N	218.7 ± 42.2	1.30 ± 0.20	0.34 ± 0.06
N_t	237.0 ± 25.2	1.37 ± 0.14	0.25 ± 0.06
	n.s.	n.s.	n.s.
T	201.7 ± 32.1	1.13 ± 0.19	0.23 ± 0.03
T_t	208.8 ± 32.4	1.18 ± 0.13	0.21 ± 0.05
	n.s.	n.s.	n.s.
M	182.0 ± 24.0	1.09 ± 0.15	0.27 ± 0.04
M_t	237.5 ± 34.6	1.30 ± 0.17	0.25 ± 0.04
	$p < 0.02$	n.s.	n.s.

Table 3

Figure 1 Protein and DNA content in musculus extensor digitorum longus

Protein and DNA concentrations/g wet weight of musculus extensor digitorum longus in non-exercised control rats, testosterone-treated rats and metenolone-treated rats are indicated (open bars) and compared with the concentrations measured in trained rats (filled bars). Means ± S.D. are indicated and statistically compared by Student's t-test.

n.s., not significant ($p > 0.05$)

Figure 2 Protein and DNA content in musculus soleus

Protein and DNA concentrations/g wet weight of musculus soleus in non-exercised control rats, testosterone-treated rats, and metenolone-treated rats are indicated (open bars) and compared with the concentrations measured in trained rats (filled bars). Means ± S.D. are indicated and statistically compared by Student's t-test.

n.s., not significant ($p > 0.05$)

group of animals	extensor digitorum longus		soleus	
	wet wt. (mg)	wet wt./body wt. (mg/g)	wet wt. (mg)	wet.wt./body wt. (mg/g)
N	272 ± 17	0.90 ± 0.07	221 ± 17	0.73 ± 0.08
N_t	285 ± 13	0.94 ± 0.03	213 ± 15	0.70 ± 0.04
	n.s.	n.s.	n.s.	n.s.
T	236 ± 33	0.93 ± 0.09	204 ± 16	0.81 ± 0.11
T_t	246 ± 21	0.96 ± 0.03	196 ± 17	0.75 ± 0.04
	n.s.	n.s.	n.s.	n.s.
M	259 ± 29	0.87 ± 0.04	201 ± 20	0.67 ± 0.04
M_t	233 ± 11	0.89 ± 0.06	189 ± 20	0.72 ± 0.09
	n.s.	n.s.	n.s.	n.s.

Table 4

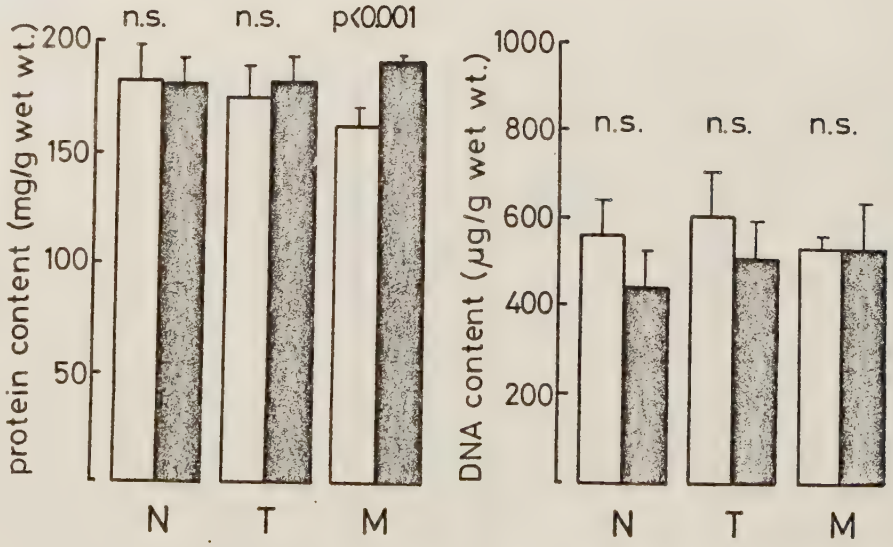
n.s.
n.s.
p<0.001
protein content (mg/g wet wt.)
200
150
100
50
N
T
M
1000
800
600
400
200
DNA content (µg/g wet wt.)
n.s.
n.s.
n.s.
N
T
M

Fig. 1

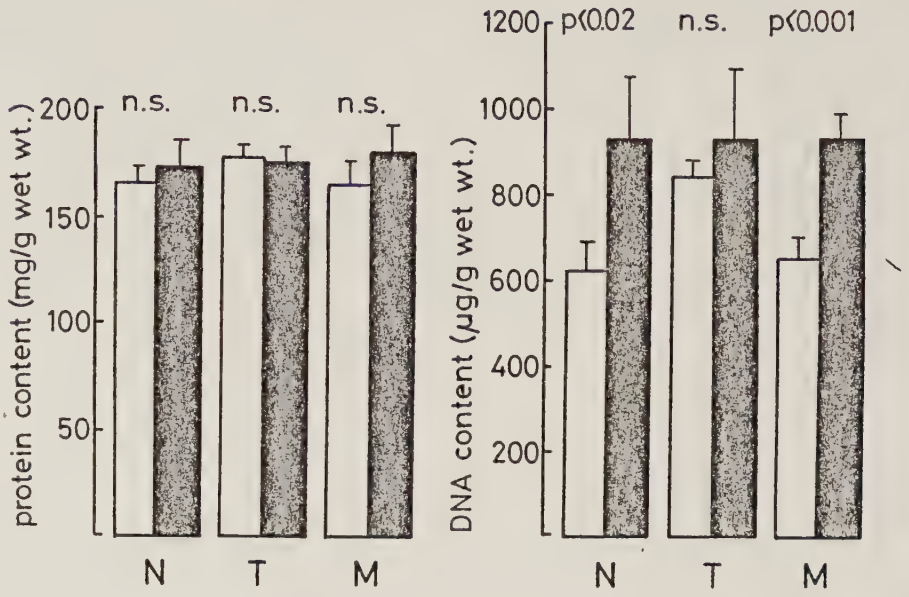
protein content (mg/g wet wt.)
200
150
100
50
n.s.
n.s.
n.s.
N
T
M
DNA content (μg/g wet wt.)
1200
1000
800
600
400
200
p<0.02
n.s.
p<0.001
N
T
M

Fig. 2

Lebenslauf

Ich bin Albertus Budiharta Widjaja, Sohn vom Ehepaar Irwan Widjaja und Mariana Tanu, geboren am 27.03.53 in Surabaja-Indonesien

1959-1965	besuchte ich die katholische Volksschule-Mittelschule-Oberschule in Surabaja
April 1972	bin ich in Deutschland angekommen
1972-1974	studierte ich an der T.H. Aachen (Sprachkurs-Studienkolleg-Physik)
1974-1980	studierte ich Medizin an den Universität Düsseldorf und Universität Essen

Am 03.06.80 habe ich das Medizinstudium abgeschlossen